REPORT OF SOME EXPERIMENTS ON THE ACTION OF STAPHYLOCOCCUS AUREUS ON THE KLEBS-LOEFFLER BACILLUS.*

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Perhaps no subject is of greater interest both to the clinician and to the bacteriologist today than the treatment of disease carriers and their relation to epidemics. The dangers and the treatment of typhoid carriers have been the subject of most searching investigations during the last few years and still we are far from having solved the question. The diphtheria carrier has not been deemed worthy of so large a place in medical literature, but is a no less serious menace to society. The question how to rid the diphtheria convalescent's throat and nose of its virulent organisms is one of supreme interest to the physician. In communities in which strict quarantine is enforced the question of getting rid of the organisms and thus raising the quarantine is of no less interest to the patients. Diphtheria antitoxin has robbed this dread disease of much of its terror, but it seems to have no power to kill the causative organisms. Antiseptics are equally powerless, and in spite of all the resources of medicine, the quarantine is frequently prolonged for days, weeks, and even months after the membrane has disappeared and the clinical symptoms would proclaim the patient well. Yet the cultures and the microscope still show bacilli, typical in form and as malignant as in the worst phases of the disease.

Graham-Smith and Cobbett's[1] tables show that while the average period of persistence in virulent diphtheria cases is 31.6 days, about one-third of the cases exceeded this limit; six of these cases lasting over 100 days.

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[1] *The Bacteriology of Diphtheria*, edited by G. H. F. Nuttall and Graham Smith, 1908, p. 421.

In my own series of 175 cases, the duration was as follows:

10	cases	terminated	in	10	days	or	less.
11	"	"	between	10	and	15	days.
40	"	"	"	15	"	20	"
25	"	"	"	20	"	25	"
35	"	"	"	25	"	30	"
33	"	"	"	30	"	35	"
7	"	"	"	35	"	40	"
6	"	"	"	40	"	45	"
2	"	"	"	45	"	50	"
1	"	"	"	50	"	60	"
2	"	"	"	60	"	70	"
3	"	"	"	70	"	80	"

From this table it may be seen that 63 per cent of the cases lasted less than 30 days, over 87 per cent of the cases lasted between 15 and 35 days, while only 12 per cent lasted under 15 days, and 12 per cent over 40 days.

It is no wonder, therefore, that the work published by Schiötz[1] in 1909, on the cure of chronic diphtheria bacillus carriers, attracted some attention. Schiötz noticed that a patient with staphylococcus sore throat failed to acquire the disease when exposed to diphtheria and also that convalescents from diphtheria in several cases ceased to give positive diphtheria cultures after an attack of ordinary staphylococcus sore throat. For these reasons he conceived the idea of treating slow convalescents with cultures of *Staphylococcus aureus*. His first patient, a young man, had been quarantined three months because cultures from his throat were constantly positive. Almost immediately after beginning the staphylococcus treatment the cultures became negative, and he was discharged on the fifth day.

The second patient, a woman of 55, cultures from whose throat had been positive for two months, gave negative cultures soon after beginning treatment and was released on the fifth day.

Four other cases were treated by Schiötz immediately after the clinical symptoms of the disease abated and with equally brilliant results.

Page[2] in 1911 reports one case in which he used the staphylococcus treatment. In Page's case, the clinical symptoms cleared

[1] *Ugesk. f. Laeger.*, 1909, 71, p. 1373; abstracted in *Jour. Am. M. Ass.*, 1910, 54, p. 422.

[2] *Arch. Inter. Med.*, 1911, 7, p. 16.

up in 14 days, but virulent Klebs-Loeffler bacilli were found in the throat cultures for about three months in spite of antitoxin, antiseptic sprays, etc.; 24 hours after beginning the staphylococcus treatment, only a few diphtheria bacilli were found, and the next day the cultures were negative and remained so for the following 15 days, during which the patient remained under observation.

Interested in these reports, I began a series of experiments to determine whether an antagonism existed between the *Staphylococcus aureus* and the Klebs-Loeffler bacillus which might account for these apparently marvelous results, or if not, whether any principles could be evolved which should place this treatment on a rational basis.

IN VITRO.

Fifteen strains of Klebs-Loeffler bacilli of different ages and varying degrees of virulence were isolated and planted on tube slants. Several methods were used to isolate the organisms, but two methods gave the best results: (1) About one part of human blood serum was added to 10 parts of plain or glycerin agar, inoculated, and poured on large plates and incubated. The colonies were then fished and transferred to serum tube slants. (2) The water of condensation of a serum tube was inoculated with a loop of mixed culture. Dilutions were made down through four or five tubes of serum. The tubes were laid down for several minutes in such a way that the slant surface was washed by the inoculated fluid. They were then placed upright in the incubator over night and the discrete colonies fished the next morning.

In the first seven sets, large serum tube slants and also blood-glycerin-agar tube slants were used; since it was found that the results in the two media corresponded exactly, only serum tubes were used in the last eight sets. Four tubes of each medium were used for each set. Tube 1 was inoculated with the diphtheria organisms alone. Tube 2 was spread with a mixture of about equal parts of staphylococcus and the diphtheria organism. Tube 3 was planted with the diphtheria culture and after 24 hours' incubation was spread with staphylococcus culture. Tube 4 was smeared with staphylococcus culture and after 24 hours' incubation the culture was killed by heating and a diphtheria

culture was then planted. This last tube was intended to show whether the staphylococcus in its growth produced any chemical substances or any chemical changes in the medium which would prevent the subsequent growth of the diphtheria bacillus. The results of these test-tube experiments were absolutely uniform and showed that in test-tubes at least there is no antagonism between these two organisms; that they grow well together, sometimes one and sometimes the other gaining a slight ascendency, and that a killed staphylococcus culture forms a good medium for the growth of diphtheria bacilli.

IN VIVO.

In the following animal experiments various methods were used to test the action of the staphylococcus upon the Klebs-Loeffler bacillus and to determine whether either organism was antagonistic to the other when grown upon animal tissue. In the first eight sets, not much attention was paid to the taking of cultures during the life of the animal, the clinical symptoms and duration of life being the main criteria of the severity of the disease process. A brief account of these experiments follows:

Set 1. The culture was taken from a two-day case. Five guinea-pigs were used. No. 1 was injected in the groin with 0.5 c.c. of a 48-hour broth culture. Slight fever developed, lasting several days. There was swelling, heat, and tenderness at the point of inoculation. This broke and discharged on the sixth day, and after this the pig rapidly improved and was killed on the ninth day. No diphtheria organisms were recovered.

Guinea-pig 2 received an injection of 0.5 c.c. of a 48-hour broth diphtheria culture and 0.5 c.c. of a staphylococcus culture simultaneously. There was marked fever and local swelling and the pig died on the third day. Cultures from the site of inoculation showed only staphylococcus and no diphtheria bacilli.

Guinea-pig 3 received an injection of 0.5 c.c. of the diphtheria culture and 24 hours later the same amount of staphylococcus culture. This pig showed about the same symptoms as No. 1, and, like it, was killed on the ninth day. Staphylococcus only was recovered from the site of inoculation.

Guinea-pig 4 had its tongue scratched and swabbed with diphtheria culture. There was a slight illness and a membrane formed but the animal recovered in a few days.

Guinea-pig 5 received the same treatment as No. 4 and 24 hours later the infected area was swabbed with staphylococcus culture. This animal was sicker than No. 4 but apparently recovered after a few days. On the 20th day after inoculation, however, he suddenly died and diphtheria bacilli were recovered from lymph glands, and pharynx and larynx.

Set 2 was from a case of four weeks' duration. The five guinea-pigs of this set

were treated in the same way as those of set 1, except that in 4 and 5 a small area on the back was excoriated and swabbed instead of the tongue. All of the animals of this set were very sick and died before the end of the fourth day. The pigs receiving staphylococcus also were somewhat sicker and died sooner than those having only diphtheria cultures.

Set 3 was from a three-week case. Only two guinea-pigs were used. In both, a small area on the back was shaved and excoriated and swabbed with diphtheria culture. Twenty-four hours later guinea-pig 2 was swabbed with staphylococcus, the latter proceeding being repeated daily. No. 2 showed less fever and swelling than No. 1, but became much emaciated. No. 1 died on the fifth day and diphtheria bacilli were recovered from the wound. No. 2 was killed on the 14th day and no diphtheria bacilli were recovered, the culture being pure staphylococcus.

In set 4, which was derived from a six-week case, two rabbits were used, a small area on the back being excoriated and swabbed with the diphtheria culture. The next day rabbit 2 was swabbed with *Staphylococcus aureus* culture and each day thereafter. Neither rabbit showed severe symptoms and 10 days later the crust was removed and the backs reswabbed with the same cultures. Rabbit 2 died under the anesthetic. Rabbit 1 developed high fever and great swelling and edema and was treated with staphylococcus four days after the second inoculation and daily thereafter. He began to improve at once and 10 days after the second inoculation, cultures showed only staphylococcus, and the rabbit was discharged.

The culture used in set 5 was from a three-week case. Rabbits were again used and this time a clean, superficial incision was made in the back and four drops of broth culture introduced into the fresh cut. Rabbit 2 was treated daily with staphylococcus culture. There was fever and local swelling for about nine days in No. 1, and these symptoms lasted longer and were more severe in No. 2.

Set 6 was from a three-week case. Two rabbits were treated as in set 5. In both there was a slight fever and local induration around the incision. Both were about equally sick and for about the same time, but there was no growth on the last three cultures in No. 1, while in No. 2 staphylococcus developed on the cultures and there was still swelling and induration.

Set 7 was also a three-week case. Two rabbits were treated as in the last two sets. Rabbit 1 had a slight fever and swelling for a few days, but was healed and showed no growth on cultures by the 12th day and was therefore discharged. Rabbit 2, which received the staphylococcus treatment, had more fever and more marked local symptoms and also became much emaciated. It developed an abscess in the neck. Staphylococcus was still present in cultures from the wound on the 12th day.

Set 8 was also from a three-week case. Two guinea-pigs were inoculated in the same way as the rabbits in the last three sets. Guinea-pig 1 had very slight general and local symptoms, but diphtheria bacilli still persisted in the wound on the 20th day, although the pig had been apparently well for one week. Guinea-pig 2 showed about the same symptoms as No. 1 but became much emaciated. The diphtheria bacilli had disappeared by the 20th day.

In the next 12 sets daily or nearly daily cultures were taken in an effort to see whether the diphtheria bacilli disappeared from the wound any more quickly when treated daily with staphylococcus than when left untreated. In each of the 12 sets, three animals were used; a guinea-pig was injected with 2 c.c. of broth culture to test virulence and two rabbits were treated locally as follows: A small area on the

back was shaved, a superficial incision was made in the skin, and the skin was then dissected from the underlying tissue, thus making a pocket, perhaps one-quarter of an inch deep. This served to protect the inoculated area from contamination with hay bacilli and other organisms. Unfortunately many of the rabbits used in this series were afterward found to have been infected with another organism, which caused large foul abscesses and interfered to some extent with the cleanliness of the wound, and reliability of the results. In all of these rabbits, pockets were made as described and two loops from a serum tube were well mixed with the serum exuding from the tissues. Rabbit 2 of each set received daily treatments with a fresh broth culture of staphylococcus, and daily cultures were taken from both rabbits; after several cultures failed to show diphtheria bacilli, the animals were discharged as cured.

The first set of this series was from a two-day case. The guinea-pig died in 24 hours. Rabbit 1 had severe general and local symptoms and died on the fourth day, all cultures showing pure diphtheria bacilli. Rabbit 2 was also very sick at first, but improved after staphylococcus was used. The cultures changed in two days from pure diphtheria to pure staphylococcus and the rabbit recovered and was discharged on the 20th day, after numerous negative cultures.

Set 2 was a three-day case. The guinea-pig died on the fifth day and pure Klebs-Loeffler bacilli were recovered from the wound. Rabbit 1 also died on the fifth day and gave a pure diphtheria culture from wound. Rabbit 2 had much less severe symptoms and gave positive diphtheria cultures for four days. Afterward the cultures were always negative and it was discharged on the 16th day.

Set 3 was a 26-day case. The guinea-pig died on the sixth day. Rabbit 1 had slight general symptoms, but the local symptoms were pronounced and complicated by a *Staphylococcus albus* infection. The cultures were positive for diphtheria for five days. Rabbit 2 was much better than No. 1. Cultures were positive for diphtheria only three days. Afterward they were always negative and it was discharged on the 16th day.

Set 4 was a very virulent two-day strain and all the animals died within 48 hours. No difference was therefore noted.

In set 5 the guinea-pig was ill, but recovered. Rabbit 1 gave positive diphtheria cultures for six days. No. 2 had a much more marked local disturbance and retained the diphtheria bacilli until the 11th day. This rabbit was left with an abscess filled with white creamy pus.

In set 6 the guinea-pig died on the 18th day. Rabbit 1 had an infection and developed an abscess. The cultures from rabbit 1 ceased to show diphtheria bacilli after the third day while those of rabbit 2 never showed diphtheria bacilli.

Set 7 was from a 14-day case. The guinea-pig died in about 25 hours. Rabbit 2 died at the end of 48 hours, but staphylococcus only was recovered from the wound. Rabbit 1 died on the eighth day and no growth developed on the cultures.

In set 8 the guinea-pig died on the first day, while rabbit 2 died on the sixth day. Rabbit 1 showed but slight symptoms and was discharged on the 11th day.

In set 9 the guinea-pig died on the second day and both rabbits showed very severe symptoms, but recovered. Rabbit 1, however, developed an abscess. Cultures from No. 1 were positive for 10 days and those from No. 2 for eight days. The local condition in No. 2 was not so severe as in No. 1.

In set 10 the guinea-pig died on the second day and there was but little difference between the rabbits, as both had had a previous infection.

In set 11 the guinea-pig died on the first day and rabbit 2 died on the 10th day with diphtheria bacilli still present in the wound and in the heart blood. Rabbit 1 had very severe general and local symptoms and cultures were positive until the 10th day.

In set 12 both rabbits died on the second day, while the guinea-pig was discharged well on the 14th day. In the last series of four cases, the pocket inoculation method was used, but instead of the loops, a thin emulsion was made by mixing one small loop of culture with about 10 c.c. of sterile broth, and giving only a small amount, 1 c.c. being given to rabbits and two to three drops to guinea-pigs. Very little difference could be seen between those animals in which staphylococcus was used and those in which it was not. In three animals the diphtheria cultures remained positive longer in the animals treated with staphylococcus than in those not so treated, while in two cases, the treated animals became negative earlier than the untreated and in one case the time was the same.

All animals that died were examined as soon as possible after death; cultures were taken and tissues were fixed in Zenker's solution and sections cut. The tissue under the point of inoculation in all cases showed edema which was often hemorrhagic; sometimes inflammatory and degenerative changes were seen. One of the cases which died late showed granulation tissue.

The kidneys were affected in all cases except one. The changes varied from slight hyperemia to acute nephritis in some cases with hemorrhage. The adrenals in all cases were enlarged, dark red in color, and dripping with blood. Microscopic sections showed marked hyperemia throughout, severe hemorrhages especially in the central portion of the gland, often an outer narrow rim of gland tissue being the only normal portion of the gland. The cells in the central portion were usually more or less degenerated. The heart was examined in only a few cases. In those examined, a slight degree of fatty degeneration was found in five cases.

Of the 32 animals inoculated with diphtheria culture and afterward treated with staphylococcus culture, nine were apparently not influenced by the treatment, 14 were worse, and nine were better than the untreated diphtheria cases. Twenty-six and two-tenths per cent were therefore neither better nor worse than if they had received no staphylococcus culture, 41 per cent were in some ways worse, while only 32.5 per cent were better than the untreated diphtheria cases. Of the animals from which cultures were taken, however, the results are slightly different, inasmuch as 40 per cent retained the diphtheria bacilli a shorter time than the untreated cases, while 28 per cent retained them longer and 31 per cent for the same time.

So far, therefore, as these experiments go, there would seem to be no rational basis for treating diphtheria cases with *Staphylococcus aureus* culture. The two organisms grow together perfectly com-

fortably on artificial media. In the animal tissues while the diphtheria organisms frequently disappear more quickly in the treated than in the untreated animals, even this result is not to be relied upon and the clinical symptoms are often much more severe under the staphylococcus treatment. Moreover, in 28 per cent of the cases the diphtheria bacilli persisted longer in the treated than in the untreated animals.

It must be remembered, however, that it has been impossible in these experiments to reproduce at all exactly the conditions in the throat of a diphtheria convalescent, as these cases are all of necessity acute cases; both cultures are implanted on a raw, bleeding surface, instead of on a surface protected by an intact mucous membrane; and we have not in the skin a natural flora to assist us in our work. We must not, therefore, draw too stringent conclusions from the animal experiments alone.

I have been so fortunate as to be allowed to try the treatment on two human patients over whom it was possible to exercise almost perfect control. The histories of these cases are as follows:

1. A woman of 19 years.—August 1, 1911, she complained of sore throat of two days' duration. The symptoms were moderately severe; both tonsils were covered with membrane and the uvula was swollen.

The cervical lymph glands were enlarged and tender. Antitoxin was at first refused but 5,000 units were given August 3, i.e., on the fifth day of the disease. From this time all symptoms improved and the membrane grew smaller. Salicylates and benzoates were given internally and the throat was sprayed with phenol and iodin. Later lactic acid milk was used as a gargle. August 14 the throat was clean and the pulse and temperature normal. Cultures still continued positive for diphtheria, however, and August 25, Dr. Schlenker, who had charge of the case, consented to try treatment with staphylococcus culture. The only pure culture which I had at hand was a *Staphylococcus albus* from a case of chronic furunculosis. The day before beginning treatment the culture from the throat was a pure virulent diphtheria culture. The method used is that recommended by Schiötz and Page: A fresh staphylococcus culture in broth was made each morning, no culture over 12 hours old being used. The throat was well swabbed out with this culture by the physician. The rest of the culture was placed in an atomizer and the patient was told to spray her throat with it well every two hou rs during the day. A culture was taken each morning before beginning the day's treatment. August 26 and August 27, the cultures were negative. The treatment was then discontinued for 24 hours and the cultures taken the next two days showed a few diphtheria bacilli; because of the two negatives, however, the case was released from quarantine and no further cultures obtained until September 5; cultures taken on that date and also September 8 and 10 showed no

diphtheria bacilli. Many staphylococci appeared in the culture, but no local or general symptoms developed after the treatment. This case is not as conclusive either way as could be desired because of the reappearance of the Klebs-Loeffler bacilli after the two negatives had been secured and because five days were then allowed to elapse without treatment before the next negative was secured. The bacteriological history of this case is so interesting and suggestive that I append it here:

August 3—Diphtheria bacilli (sick four days).
" 8— " "
" 12— " " and many staphylococci.
" 13— " " " " "
" 15— " " " few staphylococci.
" 16— " " " staphylococci equal.
" 17— " " " few staphylococci.
" 19— " " " many staphylococci.
" 21— " " " staphylococci equal.
" 21— " " pure (another culture).
" 22— " " nearly pure.
" 24— " " " "
" 25— " " " "
" 25—Staphylococcus treatment begun.
" 26—Mostly staphylococci, no diphtheria bacilli, staphylococcus culture given.
" 27—Mostly staphylococci, no diphtheria bacilli, staphylococcus culture given.
" 28—Mostly staphylococci, no diphtheria bacilli.
" 29—Mostly staphylococci, a few diphtheria bacilli, staphylococcus culture given for last time.
September 5—Staphylococci and other organisms.
" 8— " " " "
" 10— " " " " (sick 42 days).

2. Boy about 12 years of age.—He appeared at the hospital July 12, 1911, complaining of a sore throat which had lasted about four or five days. On examination, both tonsils were found tremendously swollen, so that the throat was almost occluded. The tonsils were covered by a thick yellowish exudate resembling pus. More posteriorly, however, a small patch of membrane could be seen and cultures were taken from this. Eleven thousand units of antitoxin were given in two days, after which the membrane disappeared. Potassium chlorate was given as a mouth wash and later buttermilk was also used. In spite of this, the cultures continued to be positive and on the 49th day of the disease, a pure diphtheria culture was obtained, 0.5 c.c. of which killed a rabbit on the fourth day. The staphylococcus treatment was therefore advised and begun September 2, the 58th day of the disease. The same method was used as in case 1. The cultures taken September 3 and 4 were still positive, but showed only a few diphtheria bacilli and on the fifth became negative. After four negatives had been secured the patient was discharged, but came back at intervals during the next two weeks to have cultures taken. All these cultures were negative. No injurious effects were noticed and indeed the patient said he felt much better after the treatment was begun. The staphylococcus used in this case was much more virulent than in case 1 and developed yellow pigment on the culture media.

The history of this case is as follows:

July	12—Mostly staphylococci, a few diphtheria bacilli (sick five days).
"	14— " " " " " "
"	23— " " " " " "
August	3—Staphylococci and diphtheria bacilli about equal.
"	8— " " " " " "
"	9—Staphylococci, few, mostly diphtheria bacilli.
"	12—Diphtheria bacilli almost pure.
"	19—Diphtheria bacilli and staphylococci about equal.
"	25—Diphtheria bacilli, almost pure, few staphylococci.
"	29—Diphtheria bacilli, pure.
September	2—Staphylococcus treatment started.
"	3—Staphylococcus mostly, a few diphtheria bacilli, staphylococcus culture given.
"	4—Staphylococcus mostly, a few diphtheria bacilli.
"	5—Staphylococcus mostly, staphylococcus culture given.
"	7—Staphylococcus, no diphtheria bacilli, staphylococcus culture given.
"	8—Staphylococcus, no diphtheria bacilli, treatment stopped.
"	9—Staphylococci, no diphtheria bacilli.
"	11—Culture negative and patient discharged (67th day).

Here again we have a staphylococcus throat on which diphtheria developed. The diphtheria bacilli, however, soon gained and then kept the ascendence.

So far as conclusions can be drawn from two cases, these two seem conclusive, were it not for the fact that quite frequently pure, positive cultures of long standing change to negative almost as abruptly without any treatment. Before definite conclusions can be drawn as to the value of this treatment many cases must be treated. During the coming winter, I hope to have opportunity to treat a large number of obstinate cases in this manner and a further report will then be made. Supposing, however, as seems probable from the seven cases previously reported and from my own two, that the staphylococcus may be depended on to clear up these chronic diphtheritic throats, what rational basis can be assigned for this action? Schiötz bases his claim on the failure of a staphylococcus throat to take the diphtheria infection when exposed and on the fact that some cases lost their diphtheria bacilli during a staphylococcus sore throat. This suggests a natural antipathy between these organisms. This theory seems untenable as in a series of 45 cases nearly every case began as a mixed staphylo-

coccus and diphtheria infection, and many of them were thus mixed throughout their entire course, while in the cases which became pure diphtheria culturally, the terminal cultures usually showed a gradual return of the staphylococci.

My test-tube experiments overthrow the idea of any chemical incompatibility.

It seems not to be a question of overgrowth of the area and crowding out of the diphtheria organisms, since in both the cases reported the terminal negative cultures gave but a light staphylococcus growth. The same objection applies to the inhibition explanation.

My own explanation of the action is based on cultures which I recently took from 12 normal throats, in all of which the staphylococcus was found the predominant organism, while in a few it occurred in pure or nearly pure culture. In nearly all the 45 cases of my series the first cultures showed mostly staphylococci with a few Klebs-Loeffler bacilli. In the more virulent cases the diphtheria bacillus gradually gained the ascendency and finally was the only organism found, the others being possibly killed or inhibited by the antiseptics used to destroy the diphtheria organisms. Later, as the diphtheria bacillus lost its virility, the staphylococci gradually came back, regained the ascendency, and finally were the only organisms found. In a certain few cases, however, the staphylococci were too few or the throat had taken on a reaction unfavorable to their growth and in these cases the growth of the friendly staphylococci might be favored by planting on the surface a young active culture.

SUMMARY AND CONCLUSIONS.

1. Test-tube experiments with 15 different strains of Klebs-Loeffler bacilli show that there is no inherent antagonism or incompatibility between *Staphylococcus aureus* and the Klebs-Loeffler bacillus.

2. Experiments on animals with 24 different strains of diphtheria organisms show that in 40 per cent of the cases the animals treated with *Staphylococcus aureus* got rid of their diphtheria organisms more quickly than did the untreated animals, while in 60 per

cent the cultures gave Klebs-Loeffler bacilli either the same time or longer. The clinical symptoms on the other hand were less severe in only 32.5 per cent of the animals treated with staphylococcus; as in the six patients treated by Schiötz and the one by Page, the throat culture in my cases which had been persistently positive for Klebs-Loeffler bacilli quickly became negative.

3. It would therefore seem advisable and effective to treat slowly convalescing diphtheria cases and diphtheria carriers with *Staphylococcus aureus* culture.

4. The animal experiments would indicate that a certain percentage of acute cases might clear up more quickly under the staphylococcus treatment than under the ordinary antiseptic treatment.

5. The animal experiments, however, also indicate that it would not be always altogether safe and frequently not successful to use the staphylococcus treatment before the mucous membrane of the throat has become intact so that it may protect the patient against invasion of the deeper tissues by the pyogenic organisms; hence it seems to me the treatment should not be used in acute cases, as Page advises, but only in those in which the diphtheria bacilli persist after the throat has healed and the patient is clinically well.

6. Two negative cultures are not always sufficient for a discharge, especially after this treatment, as the diphtheria bacilli may reappear after an apparent disappearance.

7. The reason for the apparently favorable action of *Staphylococcus aureus* on chronic diphtheria cases seems to be, not an antagonism or incompatibility between the two organisms, but an effort to reinforce the favorable, friendly throat flora, in the cases in which they are unable to regain their natural, normal ascendency.

In conclusion I desire to express my gratitude to Dr. D. L. Harris for support and interest during the progress of this investigation and to Dr. Florence Evans for assistance, especially in the technical part of the work.

NOTE.—Just as this paper is going to press, a brief clinical report by Drs. Catlin, Scott, and Day appeared in the *Jour. Am. M. Ass.* of October 28, 1911. These physicians treated with *Staphylococcus aureus* eight contact cases, nurses who had diphtheria organisms in their throats after taking care of diphtheria patients, but who did not develop the disease. No harmful condition developed as a result of the introduction of the staphylococci, and cultures from the treated throats quickly became negative for diphtheria bacilli.